REMARKS

Claims 17, 19, 20, 21, 23, 24, 28, 29, 31, 33, 34, 38, 41, 43, 49, 56, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85 and 86 are pending. Claims 17, 19, 20, 21, 23, 24, 28, 29, 31, 33, 34, 38, 41, 43, 49, 56, 59, 60, 61, 62, and 63 have been canceled. Claims 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85 and 86 have been added. No new matter has been entered. Applicant believes no new issues are presented by the claim amendments.

Claim Amendments

New independent claims 64 and 66 have been added, reciting the limitations of newly cancelled claims 17 and 19, respectively, but with the recitation "oligonucleotide being specific only for RNA encoded by said gene, and/or for cDNA complementary to RNA encoded by said gene, in said samples" being replaced with the recitation "oligonucleotide being specific for RNA encoded only by the gene, and/or for cDNA complementary to RNA encoded only by the gene, in the samples". Specification support for this amendment can be found throughout the specification.

New independent claims 65 and 67 have been added, reciting the limitations of newly added claims 64 and 66, respectively, with the recitation "blood samples which have not been fractionated into cell types" being replaced with the recitation "samples of whole blood". Specification support for reciting samples of "whole blood" can be found throughout the specification of the published application, and, for example, at the paragraph spanning pages 59–60 of the parent provisional application.

New dependent claims 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85 and 86 have been added, reciting the limitations of newly cancelled claims 20, 21, 23, 24, 28, 29, 31, 33, 34, 38, 41, 43, 49, 56, 59, 60, 61, 62 and 63, respectively, but with dependency to claim 17 having been replaced with alternate dependency to claim 64 or 65, and with dependency to claim 19 having been replaced with alternate dependency to claim 66 or 67. Additionally, the recitation "candidate markers" in claim 62 has been replaced with the recitation "potential markers" in new claim 85. Specification support for reciting "potential" markers can be found,

for example, at paragraph [0013] of the published application (Pub. No.: US 2007/0031841 Al), and at the Abstract and page 59 of the parent provisional application (US Patent Application No. 60/115,125).

Priority

The Examiner has indicated that the claims have basis in parent applications 10/268,730 and 09/477,148, and thus have an effective filing date of at least 1/4/00, but contends that the provisional application 60/115,125 does not provide inherent basis for the limitation that the blood samples have not been fractionated into cell types on the grounds that it does not provide express support for this limitation. While disagreeing that the provisional application does not provide adequate basis for this limitation, in the interest of expediting prosecution Applicant has added new independent claims 65 and 67 which recite the limitation "samples of whole blood" which finds express support in the parent provisional application, as described above. In view of this amendment, Applicant submits that at least claims 65, 67 and all claims depending therefrom have an earliest filing date corresponding to the filing date of the provisional application, i.e. January 6, 1999.

Claim Rejections – 35 USC § 103(a)

Claims 17, 19, 20, 21, 23, 24, 28, 29, 31, 33, 34, 38, 41, 43, 49, 56, 61, 62, and 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sharma et *al.* (WO 98/49342) in view of Ralph et *al.* (US 6109857 and WO 98/24953).

(1) The Office Action upholds the contention, previously set forth in the Office Action dated 9/17/08 ("previous Office Action"), that Sharma *et al.* teaches a method for identifying a marker useful for diagnosing a disease involving detecting and quantifying RNA in an unfractionated sample of whole blood from one or more subjects having the disease, based on the pivotal assertion that isolation of RNA from whole blood is adequately taught specifically at p. 35, section 5.1.1 (referred to herein as "cited section 5.1.1"). In Applicant's response to the previous Office Action, Applicant argued that the ordinarily skilled artisan would not be motivated to combine the cited teachings of Sharma *et al.* with any other teachings in the art,

including those of Ralph *et al.* (WO 98/24935) or Ralph *et al.* (US6190857) (collectively referred to herein as "Ralph *et al.*"), so as to arrive at the claimed invention since the artisan would not consider the teachings of Sharma *et al.* to be generally credible and reliable, and would not consider the cited combination of teachings to enable the claimed invention. In particular, in the response to the previous office action, Applicant pointed out that Sharma *et al.* clearly fails to teach a critical element of a method for enabling isolation of RNA from whole blood, namely addition of ribonuclease inhibitor to blood samples during the freezing, thawing, and initial centrifugation steps, during which such addition is necessary to prevent widespread degradation of RNA, as taught by Kephart (Promega Notes Magazine Number 62, 1997) cited in the Office Action dated 08/11/2006. The present Office Action states that it was settled that at the time of the invention the addition of a ribonuclease inhibitor would have been necessary, or at least preferable, in the process taught by Sharma *et al.*

Applicant firstly wishes to respectfully point out that the Office Action's assertion that addition of ribonuclease inhibitor could be considered merely preferable in the context of the blood freeze-thaw method taught by Sharma is a clear mischaracterization of Applicant's arguments and of the unambiguous and explicit experimental teachings of the prior art. The necessity for addition of ribonuclease inhibitor during the blood freeze-thaw process is explicit and unambiguous in view of the recitation: "*As shown in Fig. 3, the production of... amplified beta-actin product was completely dependent on the inclusion of ... Ribonuclease Inhibitor in the reaction tube during the freeze-thaw cycle*" (Kephart, section titled "*RT-PCR with RNA isolated from human blood using a freeze-thaw protocol*", paragraph starting at bottom of page, and from Figure 3 itself which shows a complete absence of amplification product in lane 1 corresponding to the sample subjected to a freeze-thaw cycle without added ribonuclease inhibitor). Applicant wishes to point out that Kephart constitutes particularly convincing evidence of the necessity for ribonuclease inhibitor during the freeze-thaw phase of the method taught at cited section 5.1.1, since the target mRNA in Kephart is beta-actin, which is so abundant as to possibly be the most abundant mRNA in all non-muscle cells, in accordance with the recitation: "*In all nonmuscle mammalian cells, beta-actin is one of the, if not the, most abundant mRNAs.*" in Gunning *et al.* (of record) 1st paragraph of "Discussion" section. It logically follows that addition of ribonuclease inhibitor is necessary, and not simply preferable, if even the maximally abundant beta-actin mRNA cannot be detected without addition of ribonuclease inhibitor during the

freeze-thaw cycle. Thus, Applicant respectfully submits that in view of the plain language of the Kephart experimental evidence, it was settled at the time of the invention that addition of ribonuclease inhibitor was clearly necessary, and not simply preferable, to enable the process taught at cited section 5.1.1, upon which the Office Action's rejections are pivotally based. Moreover, it clearly follows that since the prior art teaches that beta-actin mRNA, which is maximally abundant, is undetectable without addition of ribonuclease inhibitor during the freeze-thaw cycle, then the prior art all the more convincingly teaches that it would not be possible to comparatively quantitate differing levels of any other, inherently less abundant, mRNA for identification thereof as a disease marker in accordance with the claims, in the absence of ribonuclease inhibitor during the freeze-thaw cycle as taught at cited section 5.1.1. Indeed, it logically follows that even if the prior art hypothetically taught that beta-actin mRNA, which is maximally abundant, could be detected even at minimal threshold levels, then the prior art would still inherently teach that it would not be possible to detect, and all the more so comparatively quantitate, differing levels of any other, inherently less abundant, mRNA for identification thereof as a disease marker in accordance with the claims, in the absence of addition of ribonuclease inhibitor during the freeze-thaw cycle as taught at pivotally cited section 5.1.1 of Sharma *et al.*

With regard to the teachings of Sharma *et al.*, the Office Action further pivotally contends that a putative silence of this reference concerning addition of ribonuclease inhibitor in the detailed method taught at cited section 5.1.1 is insufficient to establish that the disclosure is not enabling, on the grounds that this would have been well known in the art at the time the invention was made, and preferably omitted from their specification.

Applicant submits that it can be shown, however, in accordance with the following clarifications, that the aforementioned contention represents a misinterpretation of the teachings of cited section 5.1.1 when properly considered as a whole.

Firstly, cited section 5.1.1 plainly teaches in the first paragraph that each blood sample is "*divided into two aliquots which are immediately frozen in liquid nitrogen*". This is clearly an explicit and unambiguous teaching, in plain language, to "immediately" freeze primary blood samples, i.e. without modifying these in any way, such as by addition of ribonuclease inhibitors, and inherently to also subsequently thaw the unmodified frozen blood samples in the absence of

ribonuclease inhibitor, particularly in view of the fully detailed and comprehensive nature of the teachings of cited section 5.1.1, which explicitly take into account use of ribonuclease inhibitors, as clarified below. Applicant submits that this, in and of itself, is sufficient to establish that cited section 5.1.1 teaches a method of RNA isolation which the ordinarily skilled artisan would consider to be grossly defective, such that the artisan would consider Sharma *et al.* to be thusly generally unreliable with regard to arts relating to blood. The artisan would thusly consider the Sharma *et al.* reference further in view of the complete lack of any reduction to practice involving blood in any way in humans or animals in Sharma *et al.*, and still further in view, to the best of Applicant's knowledge, of the complete lack of any blood-related publications by either of the inventors of Sharma *et al.* As such, the ordinarily skilled artisan would not consider the combined teachings of Sharma *et al.* and any other teachings in the art, including those of Ralph *et al.*, to enable the claimed method, and would not be motivated to combine the teachings of Sharma *et al.* and any other teachings in the art, including those of Ralph *et al.*, to arrive at the claimed invention.

It can be further shown, when carefully and properly considering the teachings of cited section 5.1.1, as a whole, that the ordinarily skilled artisan would not consider the combined teachings of Sharma *et al.* and Ralph *et al.* to enable the claimed method, and would not be motivated to combine the teachings of Sharma *et al.* and any other teachings in the art, including those of Ralph *et al.*, to arrive at the claimed invention. Namely, Applicant wishes to provide the following clarifications showing that in fact the ordinarily skilled artisan would consider that cited section 5.1.1 is clearly not simply silent with regard to addition of ribonuclease inhibitor during the freezing, thawing and initial centrifugation steps as a result of deliberately omitting what is well known in the art, as alleged in the rejections. Rather, Applicant submits that the ordinarily skilled artisan would readily conclude that Sharma *et al.* teaches a method in which ribonuclease inhibitor is not required during the freezing, thawing and initial centrifugation steps in light of the fact that an otherwise comprehensively detailed description of the various chemicals used in all other method steps is provided, critically including explicit references to ribonuclease inhibitors, within cited section 5.1.1 which is exclusively and discretely dedicated to describing the RNA isolation method taught by Sharma *et al.* Namely, cited section 5.1.1 explicitly teaches use of ribonuclease inhibitors ("Solution A" containing 2-Mercaptethanol and guanidine thiocyanate, which are ribonuclease inhibitors as taught in the Abstract of Chirgwin,

of record) in multiple subsequent steps following freezing, thawing and initial centrifugation, when they are less required than during freezing, thawing and initial centrifugation, i.e. prior to removal of whole cellular contaminants during which contamination with ribonucleases is inherently maximal. It therefore logically follows that the teachings of cited section 5.1.1 selectively and deliberately omit to teach addition of ribonuclease inhibitor specifically during the freezing, thawing and initial centrifugation steps when such is critically required, as taught by Kephart, and not simply as a result of this being so well known in the art as to not require inclusion in the otherwise highly detailed protocol, as pivotally contended by the Office Action. Clearly, cited section 5.1.1 is not, as asserted by the Office Action, written in a way which can be reasonably interpreted as deliberately omitting what is well known in the art. The otherwise comprehensively detailed description of the chemical components used in the method steps following freezing, thawing and initial centrifugation include: pellet resuspension in solution A containing "*4M Guanidine thiocyanate, 25mM Na-citrate, pH 7.0, 0.5% (w/v) N-laurylsarcosine, 0.1M 2-Mercaptethanol*", addition of "*1 ml 2M Na-Acetate, pH 4*", addition of "*1 ml water-saturated phenol*", addition of "*0.2 ml of 49:1 chloroform/bromochloropropane*", addition to the upper phase of centrifuged lysate of "*1 ml (1 vol) of 100% isopropanol*", dissolution of pelleted RNA in "*0.3 ml Solution A*" (containing the ribonuclease inhibitors guanidine thiocyanate and 2-Mercaptethanol), precipitation of RNA with "*0.3 ml (1 vol) of 100% isopropanol*", RNA pellet resuspension in "*75% Ethanol*", pellet resuspension in "*100µl water*", calculation of RNA concentration in a "*1:100 dilution of the stock*", adjusting of the pH with "*conc. Na2HP04 to 1mM*". All of the aforementioned details of RNA isolation, which include reference to ribonuclease inhibitors, are included in cited section 5.1.1 despite most or all of which being well known in the prior art, as described for example, at the Materials and Methods section of Chomczynski & Sacchi, 1987, of record. Applicant wishes to point out that Sharma *et al.*, section 5.1.1 must be considered as a whole according to MPEP 2141 which states: "*When applying 35 U.S.C. 103, the following tenets of patent law must be adhered to: The references must be considered as a whole.*", and MPEP 2141.02 which states: "*A prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention.*" Applicant submits that it can be clearly concluded that the detailed teachings of cited section 5.1.1 properly considered as a whole teach a method in which the freezing, thawing and initial centrifugation steps are selectively performed without requiring

addition of ribonuclease inhibitor, i.e. when ribonuclease contamination is maximal and ribonuclease inhibitors are most necessary. Thus, in view of the necessity for addition of ribonuclease inhibitor during freezing and thawing of whole blood samples for RNA isolation taught by the prior art and in view of the whole of the detailed plain language teachings of cited section 5.1.1, which provides a fully detailed protocol, including improperly selective use of ribonuclease inhibitors, Applicant respectfully submits that the ordinarily skilled artisan would consider Sharma *et al.* to be grossly defective and non-enabled with regard to teaching fundamental aspects of RNA isolation from whole blood, and generally unreliable with regard to arts relating to whole blood RNA.

Applicant additionally submits that the ordinarily skilled artisan, at the time of the invention, would consider Sharma *et al.* to be thusly generally unreliable with regard to arts relating to blood further in view of the complete lack of any reduction to practice involving blood in any way in Sharma *et al.*, and still further in view, to the best of Applicant's knowledge, of the complete lack of any blood-related publications by either of the inventors of Sharma *et al.* As such, the ordinarily skilled artisan would not consider the combined teachings of Sharma *et al.* and any other teachings in the art, including those of Ralph *et al.*, to enable the claimed method, and would not be motivated to combine the teachings of Sharma *et al.* and any other teachings in the art, including those of Ralph *et al.*, to arrive at the claimed invention.

The Office Action contends that evidence that one could have followed the guidance of Sharma *et al.* to successfully identify differentially expressed marker genes in whole blood is provided in an unspecified Declaration filed under Rule 132, out of the two of record, which were filed by Sharma *et al.* during prosecution of their US Pat Appl No. 09/429,003, which application containing an apparently identical section 5.1.1 as Sharma *et al.*, and which Declarations teaching use of ribonuclease inhibitor during freezing and thawing of blood samples. Applicant respectfully submits that it can be clearly shown that these Declarations in fact do not provide evidence for enablement of the method taught by Sharma *et al.* at section 5.1.1. since neither of these Declarations actually follow the detailed, comprehensive and plain language guidance of section 5.1.1, pivotally cited by the Office Action, which, in accordance with the clarifications provided above, plainly teaches grossly defective and selective omission of ribonuclease inhibitor during the freezing, thawing and initial centrifugation steps, i.e. during maximal ribonuclease contamination when ribonuclease inhibitor is most required. At most,

even the earliest of the Declarations, which was filed 11/21/2002, serves to indicate a greatly delayed acquisition of some measure of credible skill in the arts relating to whole blood RNA isolation, i.e. nearly 6 years after the 04/30/97 priority date claimed by Sharma *et al.*, nearly 5 years after the 30/04/98 filing date of Sharma *et al.*, and over 3 years after the earliest filing date of 06/01/99 claimed in the present application. Thus, even if the Declarations, which are post-filing art, are interpreted as demonstrating some level of credible skill in the relevant arts, this in no way diminishes the critical relevance of the fact that the plain language of cited section 5.1.1 explicitly, comprehensively and unambiguously teaches a non-enabled method to practice the elementary process of RNA isolation from whole blood which would lead the ordinarily skilled artisan at the time of the invention to consider the cited Sharma *et al.* teachings to fail to display credible and reliable skill in the arts relating to blood RNA, all the more so in view of the complete lack of any reduction to practice involving blood in any way in Sharma *et al.*, and still further in view, to the best of Applicant's knowledge, of the complete lack of any blood-related publications by either of the inventors of Sharma *et al.* As such, the ordinarily skilled artisan would not consider the combined teachings of Sharma *et al.* and any other teachings in the art, including those of Ralph *et al.*, to enable the claimed method, and would not be motivated to combine the teachings of Sharma *et al.* and any other teachings in the art, including those of Ralph *et al.*, to arrive at the claimed invention.

Critically, the rejections are based on the contention that the ordinarily skilled artisan would not consider Sharma *et al.* to be an inherently unreliable reference with regard to arts relating to blood RNA analysis, and would be motivated to combine the cited teachings of Sharma *et al.* and Ralph *et al.* to arrive at the claimed invention, despite the plain language of the whole of cited section 5.1.1 selectively omitting to teach critical addition of ribonuclease inhibitor during freezing and thawing of whole blood samples, on the grounds that what is well known in the art is "preferably omitted" from a patent application specification (Office Action, page 3), which is a consideration that is specific to the field of U.S patent examination practice and which is used to determine the patentability of patent claims under examination in relation to their corresponding specification. Thus, the Office Action in fact effectively contends that the ordinarily skilled artisan would not interpret the teachings of Sharma *et al.* solely on the basis of knowledge of the relevant technical arts (e.g. blood RNA analysis) at the time of the invention which by very definition define the scope of the knowledge of the artisan, but rather that the

artisan would further interpret the teachings of Sharma *et al.* via inferences made on the basis of knowledge of the field of U.S. patent examination practice. The rejections are therefore effectively specifically based on the contention that the ordinarily skilled artisan would interpret the unambiguously clear selective omission of cited section 5.1.1 to teach critical addition of ribonuclease inhibitor during freezing and thawing of whole blood samples exclusively from the point of view of a person knowledgeable in the field of U.S. patent examination practice instead of from the point of view of a person having ordinary skill in the relevant technical arts.

Applicant respectfully submits that the Office Action has therefore thereby effectively applied an erroneous standard in determining how the ordinarily skilled artisan would interpret the teachings of cited section 5.1.1 so as to be motivated to combine the teachings of Sharma *et al.* and any other teachings in the art, including those of Ralph *et al.*, to arrive at the claimed invention. Applicant wishes to point out that the ordinarily skilled artisan is in fact a person having ordinary skill in the art to which the claimed subject matter pertains, i.e. the technical arts, to the exclusion of a person having ordinary skill with regard to U.S. patent examination practice, in accordance with MPEP 2141.03 which states: "*The "hypothetical 'person having ordinary skill in the art' to which the claimed subject matter pertains would, of necessity have the capability of understanding the scientific and engineering principles applicable to the pertinent art.*" and in accordance with MPEP 2141.03 which states: "*The examiner must ascertain what would have been obvious to one of ordinary skill in the art at the time the invention was made, and not to the inventor, a judge, a layman, those skilled in remote arts, or to geniuses in the art at hand.*" Applicant submits that the ordinarily skilled artisan would interpret the plain language of the whole of cited section 5.1.1 which clearly selectively omits teaching the elementary and critical addition of ribonuclease inhibitor during freezing and thawing of whole blood samples from the point of view of one having ordinary skill in the relevant technical arts. Namely, the artisan would thereby interpret Sharma *et al.* as being generally unreliable and non-enabling with regard to any teachings related to blood RNA analysis and would thereby not be motivated to combine the teachings of Sharma *et al.* with any other teachings in the art, including those of Ralph *et al.*, so as to arrive at the claimed method, all the more so in view of the complete lack of any reduction to practice involving blood in any way in Sharma *et al.*, and still further in view, to the best of Applicant's knowledge, of the complete lack of any blood-related publications by either of the inventors of Sharma *et al.*

These obviousness rejections under Sharma *et al.* in view of Ralph *et al.* are further based on the contention that Applicant "*is applying a higher standard for disclosure by Sharma et al. than is present in the instant application or any of the parents*" (page 4 of the Office Action), i.e. on grounds relating to the quantity of enabling disclosure of the instant specification or those of the parents in relation to the claimed method which is under rejection for obviousness under Sharma *et al.* in view of Ralph *et al.* Applicant respectfully submits that the Office Action has thereby effectively applied a clearly erroneous standard for determining the obviousness of the claims, i.e. for determining whether the ordinarily skilled artisan, at the time of the invention, would consider the cited teachings of Sharma *et al.* to be enabled; would consider Sharma *et al.* to be a reliable reference with regard to the relevant technical arts; would consider the combined teachings of Sharma *et al.* and other teachings in the prior art, such as Ralph *et al.*, to enable the claimed method; and would be motivated to combine the teachings of Sharma *et al.* with other teachings in the prior art, such as those of Ralph *et al.*, to arrive at the claimed method. Applicant wishes to point out that the relationship between the level of enabling disclosure of the instant specification/parents for enabling the claims under prosecution, and the level of enabling disclosure of the cited teachings of Sharma *et al.* is plainly irrelevant to the present rejections. Namely, the relationship between these levels is not a valid factor in determining the obviousness of the claims, i.e. in determining whether the ordinarily skilled artisan, at the time of the invention, would consider the cited teachings of Sharma *et al.* to be enabled; would consider Sharma *et al.* to be a reliable reference with regard to the relevant technical arts; would consider the combined teachings of Sharma *et al.* and other teachings in the art, such as Ralph *et al.*, to be enable the claimed method; and would be motivated to combine the teachings of Sharma *et al.* with other teachings in the art, such as those of Ralph *et al.*, to arrive at the claimed method. The level of enablement of the cited teachings of Sharma *et al.* for the purposes of determining the obviousness of the claims of the present application is not affected in any way by the level of enablement which the specifications of the present application/parents confer upon the claims of the present application. The level of enablement which the specifications of the present application/parents confer is only relevant for examining the claimed method under section 112, enablement, and not for examining the claims under section 103. Thus, the Office Action applies an invalid standard for examining the claims for obviousness and thereby fails to demonstrate that the claims are rendered obvious under Sharma *et al.* in view of the prior art, such as Ralph *et*

al.

Applicant notes that the Office Action asserts that the specifications of the present application/parents have not been cited as being lacking adequate guidance for enablement of the claimed methods, on grounds that at the time the invention was made, methods for isolating total mRNA from fresh or frozen samples were routine. This serves as grounds for the Office Action's contention that, at the time of the invention, one of ordinary skill in the art would have considered the cited teachings of section 5.1.1 to be enabled in such a way as to have motivated the ordinarily skilled artisan to combine the cited teachings of Sharma *et al.* and Ralph *et al.* to arrive at the claimed method. In further support of this contention, the Office Action, at page 5, has quoted the following citation of MPEP 2121 (III): "*A prior art reference provides an enabling disclosure and thus anticipates a claimed invention if the reference describes the claimed invention in sufficient detail to enable a person of ordinary skill in the art to carry out the claimed invention; "proof of efficacy is not required for a prior art reference to be enabling ....*".

Applicant firstly wishes to point out that the MPEP 2121 (III) citation is plainly inappropriate for making the present rejections since this citation is only relevant to anticipation and not to obviousness rejections, such as the present rejections. This is firstly clear from the term "anticipates" in the first part of the citation: "*A prior art reference provides an enabling disclosure and thus anticipates a claimed invention...*". Secondly, Applicant further wishes to point out that the second part of the citation set forth in the Office Action is truncated so as to critically omit the final 4 words, "***...for purposes of anticipation.***" of the citation which explicitly and unambiguously indicate that the citation is only relevant to anticipation rejections, and not to obviousness rejections, such as the present rejections, and is therefore clearly invalid to support the present obviousness rejections. Namely, the second part of the citation in its entirety is: "*proof of efficacy is not required for a prior art reference to be enabling* ***for purposes of anticipation***." Thus, the Office Action, in this instance as well, applies an invalid standard for examining the claims for obviousness and thereby fails to demonstrate that the claims are rendered obvious under Sharma *et al.* in view of the prior art, such as Ralph *et al.*

Even if the MPEP 2121 (III) citation were hypothetically appropriate to support the

present rejections, Applicant would firstly point out that the citation would only be appropriate to support basing rejections on a single prior art reference which enabled the claimed invention in its entirety, in accordance with the plain language of the citation, "*A prior art reference… which describes the claimed invention in sufficient detail to enable a person of ordinary skill in the art to carry out the claimed invention;*", which is not the present case since the Office Action has cited a combination of references as being necessary to enable the claimed invention in its entirety. Secondly, with regard to the second part of the citation, which states that "*proof of efficacy is not required for a prior art reference to be enabling*", Applicant asserts that not only do the teachings of Sharma *et al.* fail to provide "proof of efficacy" for enabling isolation of RNA from whole blood, as conceded by the Office Action, such that the combination of Sharma *et al.* and prior art teachings, such as Ralph *et al.*, enable the claimed method, but that the prior art in fact explicitly and unambiguously provides proof of inefficacy with regard the teachings of Sharma *et al.* for the purposes of enabling isolation of RNA from whole blood such that the combination of Sharma *et al.* and prior art teachings, such as Ralph *et al.*, enable the claimed method. Namely, Applicant has provided extensive clarifications, above, which clearly demonstrate that the detailed plain language teachings of section 5.1.1, properly considered as a whole, describe a method which the explicit, unambiguous and plain language of the prior art teachings of Kephart proves lacks efficacy to enable any detection of even a maximally abundant RNA such as beta-actin, i.e. such that the cited teachings of Sharma *et al.* in combination with the teachings of the prior art, such as Ralph *et al.*, fails to enable the claimed method. Thus, the Office Action, in this further instance, applies an invalid standard for examining the claims for obviousness and thereby fails to demonstrate that the claims are rendered obvious under Sharma *et al.* in view of the prior art, such as Ralph *et al.*

Thus, in view of the clarifications set forth above, Applicant submits that the cited teachings of Sharma *et al.* in combination with prior art teachings, such as Ralph *et al.*, fail to enable the claimed method. Nevertheless, in the interest of expediting prosecution, and of clarifying the differences between the subject matter which Applicant considers to have invented and the cited teachings of Sharma *et al.*, which Applicant submits is generally unreliable with respect to blood-related arts and which fails to teach RNA isolation from whole blood; and Ralph *et al.*, which fails to teach use of whole blood samples; Applicant has added new independent claims 65 and 67, and new claims depending therefrom, which limit the blood samples to whole

blood samples.

(2) The Office Action further contends that Applicant did not persuasively demonstrate in the previous response that at the time of the invention the ordinarily skilled artisan would not have had a reasonable expectation of success in achieving, as required by the claims, detection, in RNA of blood samples which have not been fractionated into cell types from control/healthy subjects, of RNA encoded by two or more genes which are differentially expressed between subjects having a disease and control/healthy subjects, for genes which have only been demonstrated to be differentially expressed in fractionated mononuclear cells between disease subjects and control/healthy subjects, as allegedly taught by the cited teachings of Ralph *et al.*

In the previous response, Applicant cited a series of references illustrating a prevailing paradigm, i.e. the major, most investigated model system, at the time of the invention for RT-PCR analysis of disease biomarkers in blood, namely analysis of liver cancer via detection of differential expression of the alpha-fetoprotein gene (afp) in blood. These references showed that while at the time of the invention detection of afp expression had been achieved in fractionated mononuclear cells (i.e. the only cell preparation taught in Ralph *et al.*) of healthy/control subjects (Ishikawa *et al.*), in contrast, afp expression had never been detected in any blood sample containing whole leukocytes, i.e. the totality of the nucleated/DNA-containing/RNA-expressing, true "cells" of blood. Namely, afp expression had repeatedly never been detected either in samples of whole blood (Funaki *et al.*, which used the most highly sensitive method of all of the cited references; and whole blood being the only cell preparation taught in Sharma *et al.*) or in samples of isolated whole leukocytes (Matsumura *et al.*, 1994; Matsumura *et al.*, 1995; and Lemoine *et al.*). In the previous response, Applicant argued that based on this fact pattern of the prior art, which relates to the most investigated model system at the time of the invention for RT-PCR analysis of disease biomarkers in blood, the ordinarily skilled artisan would not have had a reasonable expectation of success in achieving, as required by the claims, detection, in RNA of blood samples which have not been fractionated into cell types from control/healthy subjects, of RNA encoded by two or more genes which are differentially expressed between subjects having a disease and control/healthy subjects, for genes which have only been demonstrated to be differentially expressed in fractionated mononuclear

cells between disease subjects and control/healthy subjects, as allegedly taught by the cited teachings of Ralph *et al.*

The Office Action contends that all of the references cited by applicant do not support Applicant's position on the grounds that Matsumura *et al.* (1994) and (1995) both teach analysis of afp RNA in whole leukocyte preparations isolated from erythrocytes having been removed via fractionation. The Office Action also contends that Liu *et al.* cannot be considered to unambiguously support Applicant's position on the grounds that the phrase *"mRNA was amplified from total RNA extracted from whole blood"* does not unambiguously point to RNA from blood samples which have not been fractionated into cell types.

The Office Action further contends that Applicant's argument that there was a prevailing paradigm regarding identification of disease markers in blood samples which have not been fractionated into cell types is not supported on the grounds that Applicant's review of the literature is "selective, not exhaustive", i.e. that Louha *et al.* failed to detect AFP expression in mononuclear cells isolated using a density gradient, and that Komeda *et al.* failed to detect AFP mRNA in mononuclear cell-enriched samples from healthy controls, and further on the grounds that nothing in the text of Ishikawa *et al.* suggests that they believe that their detection of AFP in healthy controls was due to the method they used to process the blood samples, namely using fractionated blood samples relative to unfractionated blood samples.

Applicant respectfully disagrees with the Office Action's contention that Applicant did not persuasively demonstrate in the previous response that the ordinarily skilled artisan would not have had a reasonable expectation of success in arriving at the claimed method when combining the teachings of Sharma *et al.* and Ralph *et al.* in the manner alleged in the rejections. Applicant submits that the fact pattern of the references of the references unambiguously demonstrates that, at the time of the invention, afp RNA had only ever been detected in fractionated, or isolated mononuclear cells (Ishikawa *et al.*) of healthy controls, and had strikingly never been detected in a significant number of attempts, including maximally sensitive attempts, in any sample from healthy controls containing whole leukocytes (Funaki *et al.*, Matsumura *et al.*, 1994; Matsumura *et al.*, 1995; and Lemoine *et al.*), i.e. a sample containing unfractionated true blood cells, in particular the totality of the nucleated/DNA-containing/RNA-expressing "true" cells of blood. Applicant submits that the fact mentioned by the Office Action

that detection had not been achieved in two other attempts (Louha, *et al.* and Komeda *et al.*) in fractionated or enriched mononuclear cells is irrelevant to the fact that afp RNA had only ever been detected in such cells, which serves to fairly and reasonably set forth the thrust of Applicant's argument. With regard to the Office Action's remarks that Applicant's review of the literature is "selective, not comprehensive", Applicant is not aware of a requirement to provide a comprehensive review of the literature to demonstrate the patentability of patent claims. In upholding the rejections based on the contention that nothing in the text of Ishikawa *et al.* suggests that they believe that their detection of AFP in healthy controls was due to the method they used to process the blood samples, namely using fractionated blood samples relative to unfractionated blood samples, the Office Action is effectively, and clearly erroneously, contending that the teachings of Ishikawa *et al.* were the only means available to the ordinarily skilled artisan at the time of the invention to ascertain the reason why such detection had only ever been achieved in fractionated mononuclear cells, but had not been detected, for example, in Funaki *et al.* which analyzed whole blood samples using by far the most sensitive assay of all of the above-mentioned references (i.e. 3-step, double-nested RT-PCR method as indicated in the Abstract of Funaki *et al.*). In fact, the prior art taught that RT-PCR detection of target RNAs could be significantly more difficult to achieve in certain types of samples than others due to the presence of inhibitors of RNA isolation and/or amplification. For example, the prior art explicitly and unambiguously taught that RT-PCR detection of target RNAs was significantly more difficult to achieve in whole blood samples, such as that tested in Funaki *et al.*, than in samples from which hemoglobin (i.e. erythrocytes) and serum have been removed, such as samples of isolated mononuclear cells as taught in Ishikawa *et al.* This is thusly taught in the prior art in accordance with the following critical citation from the second paragraph of the Introduction section of Kephart: "*whole blood contains extremely high concentrations of serum proteins and derivatives of hemoglobin that can interfere with efficient RNA extraction, and subsequent amplification*". Thus, in view of these explicit and unambiguous prior art teachings, Applicant submits that the ordinarily skilled artisan would conclude that detection of afp RNA had been achieved by Ishikawa *et al.*, and not by the other cited references as a result of Ishikawa *et al.* having analyzed significantly more purified blood samples than the other references. Namely, as a result of Ishikawa *et al.* having analyzed purified mononuclear cells (i.e. containing mostly lymphocytes and a small quantity of monocytes; refer, for example, to Alberts 2002_Blood cell

proportions table, of record); and the other references having analyzed isolated either whole leukocyte samples (i.e. containing mostly granulocytes in addition to mononuclear cells analyzed in Ishikawa *et al.*; refer, for example, to Alberts 2002_Blood cell proportions table, of record) or whole blood samples, in the case of Funaki *et al.* (i.e. containing serum, erythrocytes, hemoglobin, and the entire cellular content of blood). The ordinarily skilled artisan would consider this distinction between cell preparations particularly relevant in the case of Funaki *et al.* since this reference analyzed whole blood using the most sensitive assay of the afp-related references to attempt detection of afp RNA, as described above.

Applicant yet further submits that the difficulties exhibited in the prior art in achieving detection of afp RNA even in samples of isolated mononuclear cells from healthy subjects, as demonstrated by the failure of Louha *et al.* and Komeda *et al.* to achieve such detection, only serves to highlight the particularly high level of skill in the art which the ordinarily skilled artisan, at the time of the invention, would have perceived was required to practice the claimed invention, particularly using whole blood analysis. Applicant submits that the failure of these two references to achieve detection therefore in fact supports Applicant's position that the ordinarily skilled artisan would not have had a reasonable expectation of success in arriving at the claimed method by combining the cited teachings of Sharma *et al.*, which teaches a defective method for analysis of whole blood, and Ralph *et al.* which teaches analysis of isolated mononuclear cells.

Nevertheless, in the interest of further clarifying the differences between the subject matter which Applicant considers to have invented and the cited teachings of Sharma *et al.*, Applicant has added new independent claims 65 and 67, and new claims depending therefrom, which limit the claimed samples to whole blood samples.

With regard to the newly added claims, Applicant respectfully submits that, at the time of the invention, the ordinarily skilled artisan would particularly have lacked a reasonable expectation of success in achieving, as required by the claims, detection, in RNA of whole blood samples from control/healthy subjects, of RNA encoded by two or more genes which are differentially expressed between subjects having a disease and control/healthy subjects, for genes which have only been demonstrated to be differentially expressed in isolated mononuclear cells between disease subjects and control/healthy subjects, as allegedly taught by the cited teachings

of Ralph *et al.* Applicant submits that such particular lack of expectation of success with regard to use of whole blood samples would arise in view of the unreliability of Sharma *et al.* with regard to arts relating to whole blood, as clarified above; in view of well known difficulty at the time of the invention of achieving detection of target RNAs in whole blood, for example, as taught by Kephart at the second paragraph of the Introduction section; and still further in view of the extreme difficulty in achieving any detection, or sufficiently sensitive detection, of a maximally abundant RNA, such beta-actin RNA, in whole blood samples, particularly using whole blood freeze-thawing as allegedly adequately taught by pivotally cited section 5.1.1 of Sharma *et al.*, and yet still further in light of the failure of Funaki *et al.* to achieve detection of afp RNA in whole blood despite using maximally sensitive detection methods. In light of such prior art evidence, Applicant wishes to provide a reminder of the following: "*The totality of the prior art must be considered, and proceeding contrary to accepted wisdom in the art is evidence of nonobviousness.*" (MPEP 2145).

The Examiner contends that absolute predictability is not a requirement in a rejection under 103, only a reasonable expectation of success, and that there is no evidence on the record to suggest that the use of whole blood RNA as taught by Sharma *et al.* would have been considered inoperable. Applicant respectfully submits that the above arguments and clarifications firstly clearly demonstrate that the ordinarily skilled artisan, at the time of the invention, would indeed not have had "a reasonable expectation of success" in arriving at the claimed invention by combining the teachings of Sharma et *al.* with prior art teachings such as those of Ralph *et al.* With regard to the operability of the cited teachings of Sharma *et al.*, Applicant submits that above clarifications clearly and unambiguously show that the plain teachings of section 5.1.1 of Sharma et *al.* in light of the prior art teachings of Kephart et *al.* as regards detection of even a maximally abundant RNA such as beta-actin in freeze-thawed blood do indeed demonstrate the inoperability of the teachings of Sharma et *al.*

To Applicant's knowledge, Applicant is the first to have provided the claimed methods of identifying two or more markers in whole blood that are useful for diagnosing a disease. Thus, the field of identifying two or more markers in whole blood that are useful for diagnosing a disease was clearly in its infancy. The newness of the approach of using whole blood to identify

two or more markers in whole blood that are useful for diagnosing a disease negates an obvious to try motivation, according to US case law.

> "Second, an invention is not obvious to try where vague prior art does not guide an inventor toward a prior solution. A finding of obviousness would not be obtained where "what was obvious to try" was to explore a new technology or general approach that seemed to be a promising filed of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it" O'Farrell, 853 F.2d at 903. This expresses the same idea as the KSR requirement that the identified solutions be predictable 550 I.S. at 421; see also Procter & Gamble, see F.3d at 996-97; Kubin, 561 F.3d at 1359-60.", *Bayer Schering Pharma v. Barr Labs.* (Fed Cir 8/5/09)

Applicant's claimed methods of identifying two or more markers in whole blood that are useful for diagnosing a disease were, at the time of the invention, a promising new field of experimentation, with only general guidance being provided by the art. Specifically, actual markers resulting from Applicant's claimed methods of identifying two or more markers in whole blood that are useful for diagnosing a disease, had not been previously reported to the best of Applicant's knowledge.

A determination of obviousness must be made based on what a person of ordinary skill in the pertinent art would have known at the time of filing. Applicant submits that neither the teachings in the Sharma *et al.* publication itself, as discussed above, nor the teachings of Ralph *et al.*'s experiments, provide specific guidance for Applicant's methods of identifying two or more markers in whole blood that are useful for diagnosing a disease. Without such guidance, Applicant submits a prima facie case of obviousness can not be made in such a nascent field of identifying two or more biomarkers from whole blood under *Bayer Schering Pharma*.

The Examiner contends that Kruse, et al., Chadderton et al. and MacFarlane et al. provided motivation at the time of the invention to attempt to identify gene expression markers in whole blood. However, Applicant wishes to point out that these are mere technical references representing early attempts to develop appropriate methods for performing RT-PCR analysis of whole blood. None of these references in fact attempts to identify any gene which is differentially expressed in whole blood between healthy subjects and subjects having a disease, as required by the instant claims. Applicant submits that these references in fact support Applicant's position that the field of gene expression profiling in whole blood was still in its

infancy at the time of the invention, and that the experimental evidence in the field at the time of the invention (e.g. Kephart, Ishikawa et al. and associated afp-related references, as described herein) would in fact have led the ordinarily skilled artisan to not have a reasonable expectation of success in arriving at the claimed invention by combining the teachings of Sharma et al. with other prior art teachings, such as those of Ralph et al.

In light of the above remarks and amendments, Applicant very respectfully requests reconsideration and withdrawal of the instant rejections.

The rejection of claims 17, 20, 23, 28-29, 33-34, 41, 43, 49, and 59-63 under 35 U.S.C. 103(a) as being unpatentable over Sharma et *al.* in view of Lockhart et *al.* has been maintained as set forth in the office action mailed 9/17/08.

Applicant submits that the above clarifications demonstrate that Sharma et *al.* in combination with prior art teachings, such as those of Lockhart et *al.*, do not enable the claimed invention, and demonstrate that the ordinarily skilled artisan would not have had a reasonable expectation of success at the time of the invention in arriving at the claimed invention by combining the teachings of Sharma et *al.* and prior art teachings, such as those of Lockhart et *al.*

As described above, in the interest of expediting prosecution, and of clarifying the differences between the subject matter which Applicant considers to have invented and the cited teachings of Sharma et *al.* in combination with the prior art, Applicant has added new independent claims 65 and 67, and new claims depending therefrom, which limit the blood samples to <u>whole blood</u> samples.

In light of the claim amendments and remarks, Applicant very respectfully requests reconsideration and withdrawal of the instant rejection.

The rejection of claim 59 under 35 U.S.C. 103(a) as being unpatentable over Sharma et *al.* in view of either Ralph et *al.* as applied to claims 17 and 19 above, and further in view of Wei *et al.* is maintained as set forth in the office action mailed 9/17/08.

Applicant submits that the above clarifications demonstrate that Sharma et *al.* in

combination with prior art teachings, such as those of Ralph et *al.* and Wei et *al.*, do not enable the claimed invention, and demonstrate that the ordinarily skilled artisan would not have had a reasonable expectation of success at the time of the invention in arriving at the claimed invention by combining the teachings of Sharma et *al.* and prior art teachings, such as those of Ralph et *al.* and Wei et *al.*

As described above, in the interest of expediting prosecution, and of clarifying the differences between the subject matter which Applicant considers to have invented and the cited teachings of Sharma et *al.* in combination with the prior art, Applicant has added new independent claims 65 and 67, and new claims depending therefrom, which limit the blood samples to whole blood samples.

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As described above, in the interest of expediting prosecution, and of clarifying the differences between the subject matter which Applicant considers to have invented and the cited teachings of Sharma et *al.* in combination with the prior art, Applicant has added new independent claims 65 and 67, and new claims depending therefrom, which limit the blood samples to whole blood samples.

In light of the claim amendments and remarks, Applicant very respectfully requests reconsideration and withdrawal of the instant rejection.

The rejection of claim 60 under 35 U.S.C. 103(a) as being unpatentable over Sharma et *al.* in view of either Ralph et *al.* as applied to claims 17 and 19, and further in view of Kasuga et *al.* has been maintained as set forth in the office action mailed 9/17/08.

Applicant submits that the above clarifications demonstrate that Sharma et *al.* in combination with prior art teachings, such as those of Ralph et *al.* and Kasuga et *al.* do not enable the claimed invention, and demonstrate that the ordinarily skilled artisan would not have had a reasonable expectation of success at the time of the invention in arriving at the claimed invention by combining the teachings of Sharma et *al.* and prior art teachings, such as those of Ralph et *al.* and Kasuga et *al.*

As described above, in the interest of expediting prosecution, and of clarifying the differences between the subject matter which Applicant considers to have invented and the cited teachings of Sharma et *al.*, Applicant has added new independent claims 65 and 67, and new claims depending therefrom, which limit the blood samples to whole blood samples.

In light of the claim amendments and remarks, Applicant very respectfully requests reconsideration and withdrawal of the instant rejection.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

Date: December 3, 2009

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